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(21) International Application Number: PCT/EP97/03494 (22) International Filing Date: 27 June 1997 (27.06.97) (30) Priority Data: 96201871.9 5 July 1996 (05.07.96) EP (34) Countries for which the regional or international application was filed: NL et al. 96203643.0 19 December 1996 (19.12.96) EP (34) Countries for which the regional or international application was filed: NL et al. (71) Applicant (for all designated States except AU BB CA GB GH IE KE LK LS MN MW NZ SD SG SZ TT UG US ZW): UNILEVER N.V. [NL/NL]; Weena 455, NL-3013 AL Rotterdam (NL). (71) Applicant (for AU BB CA GB GH IE KE LK LS MN MW NZ SD SG SZ TT UG ZW only): UNILEVER PLC [GB/GB]; Unilever House, Blackfriars, London EC4P 4BQ (GB). (72) Inventors; and (75) Inventors/Applicants (for US only): VAN AMERONGEN, Mamix, P. [NL/NL]; Unilever Research Vlaardingen Lab., Olivier van Noortlaan 120, NL-3133 AT Vlaardingen (NL). LIEVENSE, Lourus, Cornelis [NL/NL]; Unilever Research		Vlaardingen Lab., Olivier van Noortlaan 120, NL-3133 AT Vlaardingen (NL). VAN OOSTEN, Cornelis, Willem [NL/NL]; Unilever Research Vlaardingen Lab., Olivier van Noortlaan 120, NL-3133 AT Vlaardingen (NL). (81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i> (88) Date of publication of the international search report: 2 April 1998 (02.04.98)
(54) Title: METHOD OF MANUFACTURING A STEROL ESTER MIXTURE		
(57) Abstract Process for the manufacture of a mixture of fatty acid esters comprising the steps of: (a) hydrolysing a sterol ester or a mixture of sterol esters such that a mixture is obtained comprising phenolic acids and/or fatty acids, and free sterols; and (b) optionally, separating the phenolic acids and/or fatty acids from the reaction mixture; and (c) esterifying the so obtained free sterols with particular fatty acids.		

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INTERNATIONAL SEARCH REPORT

Int. Application No

PCT/EP 97/03494

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C07J9/00 C11B13/02 C11B3/00 C11C3/00 A23D7/00
A23D9/00 A23L1/24

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07J C11B C11C A23D A23L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 290 579 A (HITOTSUMATSU HISASHI ET AL) 1 March 1994 see column 2, line 30 - column 4, line 10 see claims 1-3 ---	1-10, 12, 13, 15-18
X	US 4 748 161 A (KIMURA GORO ET AL) 31 May 1988 see column 11, line 59 - column 14, line 2 see examples 25.51.93 ---	1-11
Y	EP 0 503 650 A (TSUNO FOOD IND CO LTD : WAKAYAMA PREFECTURE (JP)) 16 September 1992 cited in the application see page 1, line 51 - page 2, line 48 see examples 1-5 see claims 1-10 see figure 2 ---	1-9, 12, 15-18
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☒ Further documents are listed in the continuation of box C

☒ Patent family members are listed in annex

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INTERNATIONAL SEARCH REPORT

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
Y	WO 92 19640 A (RAISION MARGARIINI OY) 12 November 1992 cited in the application see claims 1-9 ---	1-9, 12, 15-18
A	US 3 751 569 A (ERICKSON B) 7 August 1973 cited in the application see column 2, line 2 - column 3, line 63 see example I see claims 1-4, 8 ---	1, 12, 15-18
A	US 5 512 691 A (BARNICKI SCOTT D ET AL) 30 April 1996 see column 1, line 13 - line 17 see column 2, line 60 - column 5, line 2 -----	1, 7, 8, 12

INTERNATIONAL SEARCH REPORT

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International Application No

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(51) International Patent Classification ⁶ : A61K 31/22	A2	(11) International Publication Number: WO 98/01126 (43) International Publication Date: 15 January 1998 (15.01.98)
<p>(21) International Application Number: PCT/EP97/03494</p> <p>(22) International Filing Date: 27 June 1997 (27.06.97)</p> <p>(30) Priority Data: 96201871.9 5 July 1996 (05.07.96) EP (34) Countries for which the regional or international application was filed: NL et al. 96203643.0 19 December 1996 (19.12.96) EP (34) Countries for which the regional or international application was filed: NL et al.</p> <p>(71) Applicant (for all designated States except AU BB CA GB GH IE KE LK LS MN MW NZ SD SG SZ TT UG US ZW): UNILEVER N.V. [NL/NL]; Weena 455, NL-3013 AL Rotterdam (NL).</p> <p>(71) Applicant (for AU BB CA GB GH IE KE LK LS MN MW NZ SD SG SZ TT UG ZW only): UNILEVER PLC [GB/GB]; Unilever House, Blackfriars, London EC4P 4BQ (GB).</p> <p>(72) Inventors; and (75) Inventors/Applicants (for US only): VAN AMERONGEN, Marnix, P. [NL/NL]; Unilever Research Vlaardingen Lab., Olivier van Noortlaan 120, NL-3133 AT Vlaardingen (NL). LIEVENSE, Lourus, Cornelis [NL/NL]; Unilever Research</p>	<p>Vlaardingen Lab., Olivier van Noortlaan 120, NL-3133 AT Vlaardingen (NL). VAN OOSTEN, Cornelis, Willem [NL/NL]; Unilever Research Vlaardingen Lab., Olivier van Noortlaan 120, NL-3133 AT Vlaardingen (NL).</p> <p>(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).</p> <p>Published Without international search report and to be republished upon receipt of that report.</p>	
<p>(54) Title: METHOD OF MANUFACTURING AN ESTER MIXTURE</p>		
<p>(57) Abstract</p> <p>Process for the manufacture of a mixture of fatty acid esters comprising the steps of: (a) hydrolysing a sterol ester or a mixture of sterol esters such that a mixture is obtained comprising phenolic acids and/or fatty acids, and free sterols; and (b) optionally, separating the phenolic acids and/or fatty acids from the reaction mixture; and (c) esterifying the so obtained free sterols with particular fatty acids.</p>		

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Method of manufacturing an ester mixture

The present invention relates to a method for the manufacture of an ester mixture, in particular an ester mixture of
5 phytosterols and phytostanols (4-desmethylsterols, 4-monomethylsterols and 4,4'-dimethylsterols and their stanol equivalents) (hereinafter called sterols) and fatty acids.

High cholesterol levels in blood serum can cause an
10 increased risk of coronary heart diseases. Several methods have been proposed for reducing cholesterol levels.

A well-known method relates to a careful selection of fatty acids in the diet, in particular a diet rich in unsaturated
15 fatty acids can help to reduce cholesterol levels.

Another method relates to the addition of specific substances to the diet to lower the cholesterol level. For example WO 92/19640 discloses the use of β -sitostanol ester
20 for lowering cholesterol levels in serum.

It was found that particular sterol derivatives have better cholesterol lowering properties than others. A number of sterols occur in nature which are esterified. For example,
25 the majority of sterols, i.e. 25-80%, found in sources like corn, wheat, oat, rice bran and sheanut are esterified with phenolic acids such as ferulic acid, coumaric acid, caffeic acid, and cinnamic acid. However, it has been found that these esterified sterols are less suitable for
30 lowering cholesterol levels in serum.

This means that for the majority of sterol derivatives occurring in sources like corn, wheat, oat, rice bran and sheanut, there is a desire for improvement for their blood cholesterol lowering effects.

35

Further, it has been found that a significant part of sterols is not esterified at all. This type of sterols is

not easily soluble in oils, and so not very suitable as a good source for applications in fat based food products.

US 3,751,569 suggests the use of esters of monocarboxylic acid and plant sterols in dietary oils for reducing the cholesterol level.

A disadvantage of the methods of WO 92/19640 and US 3,751,569 is the high costs of the ester materials. These high costs are due to the high isolation and preparation costs for the materials.

All methods known to day for making ester materials use free sterols as occurring in nature as the starting material for obtaining ester mixtures. Also, in nature, a number of sterol esters exists that were found that are not as effective in blood cholesterol lowering as the fatty acid esters of phytosterols and phytostanols.

Where in this application sterols are mentioned, phytosterols, phytostanols or mixtures thereof are meant. Hence, the term sterols in this application refers to 4-desmethylsterols, 4-monomethylsterols and 4,4'-dimethylsterols, their stanol equivalents, and mixtures thereof in any of combination possible.

The present invention aims at providing a cheap process for the preparation of an ester mixture, said ester mixture having a cholesterol lowering effect on foods. By the presently found method, it is now possible to have all sterols and sterol derivatives present in fat rich sources, but which up till now were not suitable for obtaining sterol derivatives that have a significant blood cholesterol lowering effect. Moreover, the blood cholesterol lowering effect obtainable by the use of the total amount of sterols and sterol derivatives present in these sources was found to be increased. Thus, the amount

of effective cholelesterol lowering sterol derivatives obtainable from sources used in the past is increased, and many more sources applicable for obtaining such effective sterol derivatives have become available.

5

It has been found that the costs for producing the ester mixture can significantly be reduced if materials other than free phytosterols (US 3,751,569) or their derivatives (WO 92/19640) are used as the starting material and the
10 process involves a specific hydrolysatation step.

Further advantages of the process of the invention are that the process can be carried out without the need for high investments, the process can easily be carried out at a
15 high yield and the level of wastage is low, because the by-products can be used for other purposes.

Further advantages are that the specific ester mixture obtained by the process of the invention can very
20 conveniently be incorporated in food products at relatively high levels. They also show excellent abilities to reduce serum cholesterol levels.

Accordingly in a first aspect the present invention relates
25 to a process for the manufacture of an ester mixture comprising the steps of:

- (a) hydrolysing a sterol ester or a mixture of sterol esters such that a mixture is obtained comprising phenolic and/or fatty acids, and free sterols; and
30 (b) esterifying the so obtained free sterols with particular fatty acids.

In another aspect, the invention relates to a process for the manufacture of an ester mixture comprising the steps
35 of:

- (a) hydrolysing a sterol ester or a mixture of sterol esters such that a mixture is obtained comprising

phenolic acids and/or fatty acids, and free sterols;
and

(b) separating the phenolic acids and/or fatty from
the reaction mixture; and

5 (c) esterifying the so obtained free sterols with
particular fatty acids.

The method is very beneficial if the sterol esters are
esters of sterols and acids other than fatty acids. In this
10 embodiment, the sterol ester or mixture of sterol esters is
a mixture comprising phenolic acid sterol esters. In a
further preferred embodiment, the process is a process
wherein the sterol ester or mixture of sterol esters
consist of a phenolic acid sterol ester or a phenolic acid
15 sterol ester mixture.

In a particular embodiment the invention concerns a process
for the manufacture of an ester mixture comprising the
steps of:

(a) hydrolysing γ -oryzanol such that a mixture is
20 obtained comprising ferulic acid and sterols; and

(b) separating the ferulic acid from the reaction
mixture; and

(c) esterifying the so obtained sterols with
particular fatty acids.

25

In particular, the present invention encompasses sterol
fatty acid ester mixtures obtainable by the method of the
present invention. If in the process of the invention,
sterols of one particular source are used without
30 modification, the nature and ratio of the sterols found in
the product resulting from the process of the present
invention will be indicative of the source used. In one
embodiment of the present invention, the sterol fatty acid
ester mixtures is characterised by the nature of the type
35 of sterols found in the sterol esters and the ratio of the
different sterol, the nature and ratio being similar to
those found in the sterol derivatives present in rice bran.

In yet another embodiment, nature and ratio of the alcohol part found in the sterol fatty acid ester mixture of the invention are similar to those found in the sterol derivatives present in shea.

5

In the case there is a desire to prepare fatty acid sterol esters of a further defined fatty acid type, the process indicated above can also be applied for re-esterifying fatty acid sterol esters with a further defined group of fatty acids. With particular fatty acids is meant in this application a group of fatty acids chosen as desired.

In a most preferred embodiment of this invention, sterol esters are used that are esterified with any phenolic acid. In nature, most phenolic acid sterol esters are esters of sterols and phenolic acids from the group of ferulic, coumaric, caffeic, and cinnamic acid.

Sterol esters that are in particular suitable for the method of the present invention are the sterol esters from rice bran and from sheanut.

The sterol esters and stanol esters can be isolated from natural sources by known techniques. If so desirable, the sterol esters can be hardened to stanol esters, before or after the process of re-esterification..

A particular suitable sterol esters group to be re-esterified is oryzanol. Oryzanol or γ -oryzanol is a mixture of ferulic acid esters of mainly the phytosterols campesterol, β -sitosterol, 24-methyl-cycloartenol and cycloartenol. γ -oryzanol is present as a minor component at relatively high levels in rice-bran oil.

A very suitable method of obtaining oryzanol is described in our co-pending European Patent Application No. 96201871.9. Preferably the starting material is the by-product of the alkali refining process of rice bran oil,

the so called soap stock.

Another particular suitable sterol esters group to be re-esterified is the group of sterol esters found in sheanut,
5 a mixture of mainly cinnamic acid esters of mainly the phytosterols alpha-amyrin, beta-amyrin, lupeol, butyrospermol and germanicol.

A very suitable method method of obtaining sheanut sterols
10 is described in our co-pending European Patent Application 96304994.5.

The process now found has the further advantage that it can be applied to a mixture of sterols and sterol derivatives
15 from a particular source or a mixture of different sources. For example, a mixture can be applied as occurring in a natural source, e.g. a sterol concentrate obtained by concentrating the sterol rich part of an oil, without the need for isolation of one or more specific sterols prior to
20 subjecting it to the process of the present invention. In this embodiment, fatty acid sterol esters and phenolic acid sterol esters are re-esterified with fatty acids, and any free sterol esters present in such a composition will be esterified to fatty acid sterol esters. Accordingly, a
25 large amount or even all of the sterols present in such a natural source are processed in such a manner that they become available as effectively cholesterol lowering sterol derivatives.

30 It is preferred that the sterol ester or sterol ester mixture used in the process of the invention is of food-grade quality i.e. it is in pure form or in the form of a food-grade concentrate, for example a concentrated solution of sterol esters or of sterols comprising sterol esters in
35 a solvent e.g. a natural oil. Especially preferably the level of sterol esters in the starting mixture is from 5 to 90%, and preferably from 40-90%.

The first step of the process of the invention relates to the hydrolysis of the sterol esters. This hydrolysis is aimed at the separation of the acid group from the methylsterol group. This hydrolysis can be carried out
5 by any suitable method for hydrolysing. Suitable conditions for the hydrolysis reaction are for example described in EP 503 650. Other conditions are illustrated in the example. It is believed to be well within the ability of the skilled person to find possible variation in processing
10 conditions to carry out the hydrolysis reaction.

Preferably the hydrolysis reaction is carried out until at least 50 wt% of the sterol esters are split in the corresponding free acid and free sterols, more preferred at
15 least 80 wt%, most preferred 90 to 100 wt%. For example, in case γ -oryzanol is used, the hydrolysis reaction is carried out until at least 50 wt% of the γ -oryzanol is split in the corresponding ferulic acid and sterols, more preferred at least 80 wt%, most preferred 90 to 100 wt%

20

Suitable esters are sterol esters as found in natural sources such as wheat, corn, rice bran, oat, and sheanut. These sterol esters can be esters of phenolic acids such as ferulic, coumaric, caffeic, and cinnamic acid. The sterols
25 can be phytosterols and phytostanols (4-desmethylsterols, 4-monomethylsterols and 4,4'-dimethylsterols and their stanol equivalents), hereinafter referred to as sterols.

After hydrolysis a mixture is obtained containing one
30 or more phenolic acids and or one or more fatty acids and one or more sterols. In its preferred embodiment, the phenolic acids obtained are a by-product and not needed for further processing they could be conveniently removed from the reaction mixture. In the embodiment that fatty acid
35 sterol esters are hydrolysed, it may also be desired to remove the fatty acids obtained after hydrolysis to allow specific fatty acid sterol esters to be obtained by

esterification of the sterols with these particular fatty acids.

In case a mixture of phenolic acid sterol esters and fatty acid sterol esters (and optionally, free sterols being present) are hydrolysed, a further embodiment would also comprise the removal of any of the phenolic and fatty acids obtained by the hydrolysis process. Any of these removal steps is preferably carried out prior to esterification of the free sterols obtained with the desired particular fatty acids.

The removal can take place in any stage of the process, however for avoiding possibly occurring backward reactions with the sterols it is preferred that the phenolic acid(s) is removed from the reaction mixture before further reacting by esterification. Removal of the phenolic acid and/or fatty acid can be done by any suitable technique e.g. filtering or precipitation. EP 503 650 describes suitable methods for the removal of ferulic acid which can also be applied to other phenolic acids. Other methods are illustrated in the examples.

If the esterification is carried out under such conditions that the fatty acids are, compared to the phenolic acids, (largely) selectively esterified to the sterols, separation of the phenolic acids is not required. In this particular embodiment it may be desired to use an enzymatic (selective) esterification process.

The process of the invention also involves the esterification of the methylsterol mixture obtained in the hydrolysis reaction. Preferably the sterols are esterified with one or more C_{2-22} fatty acids. Such fatty acid groups will form an ester bond at the former attachment point of the phenolic acid. For the purpose of the invention the term C_{2-22} fatty acid refers to any molecule comprising a C_{2-22} main chain and at least one acid group. Although not

preferred within the present context the C_{2-22} main chain may be partially substituted or side chains may be present. Preferably, however the C_{2-22} fatty acids are linear molecules comprising one or two acid group(s) as
5 endgroup(s). Most preferred are linear C_{8-22} fatty acids as occur in natural oils.

Suitable examples of any such fatty acids are acetic acid, propionic acid, butyric acid, caproic acid, caprylic acid,
10 capric acid. Other suitable acids are for example citric acid, lactic acid, oxalic acid and maleic acid. Most preferred are lauric acid, palmitic acid, stearic acid, arachidic acid, behenic acid, oleic acid, cetoleic acid, erucic acid, elaidic acid, linoleic acid and linolenic
15 acid.

When desired a mixture of fatty acids may be used. For example it is possible to use a natural occurring fat or oil as a source of the fatty acid and to carry out the
20 esterification via an interesterification reaction.

In a particular embodiment, the fatty acid mixture contains a high amount (>35%, preferably >45%, further preferred >70%) of polyunsaturated fatty acids (PUFA). This
25 does not only provide the advantage of PUFA itself having good blood cholesterol lowering capacity, but also of the sterols esters prepared with such fatty acids being considered as having a higher solvability and blood cholesterol lowering efficacy in the body.

30

Preferably fatty acid mixtures of rice bran oil, sunflower, safflower, rapeseed, linseed, linola and/or soybean are used. These are typical sources of high PUFA and/or low SAFA.

35 Suitable esterification conditions are for example described in WO 92/19640. Further suitable conditions are given in the examples below.

Preferably the conditions of the esterification reaction are chosen such that at least 50 wt%, preferably at least 75 wt% most preferred from 90-100 wt% of the sterols are esterified.

5

In the particular embodiment where γ -oryzanol is used, the reaction will result in a mixture containing esters of sterols, said sterols comprising at least two sterols selected from the group of campesterol, β -sitosterol, 24-methylene cyclo-artenol and cyclo-artenol.

10

Preferably at least 50%, more preferred more than 80wt%, most preferred from 90 to 100% of the sterols is selected from the group of campesterol, β -sitosterol, 24-methylene cyclo-artenol and cyclo-artenol.

15

Especially preferably the mixture of fatty acid esters of sterols is obtained by subjecting a mixture of

- (a) 5-40 wt% campesterol
 - (b) 2-25 wt% β -sitosterol
 - (c) 20-60 wt% 24-methylene cyclo-artenol; and
 - (d) 10-50 wt% cyclo-artenol
- to the process of the present invention.

20

In another especially preferred embodiment, the mixture of esters of sterols comprises at least 50% of the sterols selected from the group of alpha-amyirin, beta-amyirin, lupeol, butyrospermol and germanicol.

25

In this embodiment a further preference exists for the mixture of fatty acid esters of sterols being obtained by subjecting a mixture of

30

- (a) 2-45 wt% alpha-amyirin
- (b) 0.2-25 wt% beta-amyirin
- (c) 0.2-35 wt% lupeol
- (d) 2-45 wt% butyrospermol and
- (e) 0.1-15 wt% germanicol

35

to the process of the present invention.

Preferably the mixture obtained by esterification is added to food products in an amount of 0.5 to 40 wt, more
5 preferred 2 to 30 wt%, most preferred 3 to 20 wt%, with a particular preference for 4-20 wt%. Surprisingly the use of these relatively high levels of the fatty acid sterol esters does generally not lead to sandiness of the product. Although applicants do not wish to be bound by any theory
10 it is believed that this reduction of sandiness may be caused by a decrease of amount and/or rate of crystallisation of the ester components compared to free sterols.

15 Especially preferably the ester mixtures of the invention are added to fat based food products. Fat based food products are food products (partially) based on fat and regarded by the user, in particular the consumer, as 'fatty type of products'. Examples are margarines, butter, yellow
20 fat spreads (containing vegetable fat and/or animal fat such as butterfat), dressings, mayonnaise, cheese, shortenings, cooking and frying oils and the like. These products in most cases comprise a particular amount of fat. In some cases, however, products are still regarded as
25 'fatty type of products', despite a replacement of part or even all the fat by fat replacers. Fat based food products in which the fat is partially or completely replaced by fat replacers are also covered by the term fat based food products of this invention.

30 Fat products consisting of fat only are also considered as fat based food products in this specification. These are not only used as cooking and frying oils, but also sometimes in the industry in the preparation of food
35 products, e.g. in baking.

The food products as such are common products in the

western world, and are used by consumers on a daily basis in amounts different for each individual.

The invention is in particular very suitable for yellow fat
5 spreads, dressings, cheese, shortenings and cooking and
frying oils, and more in particular for yellow fat spreads
which can comprise 0 (zero) to 90 wt% fat (usually 5-80
wt%). Dressings can comprise 0 to 85 wt% fatty (usually 5-
80 wt%), shortenings, cooking and frying oil more than 95
10 wt% fat. In a particular embodiment of this invention, the
fat based food product is selected from the group of
margarines, butter and spreads.

Yellow fat spreads comprising less than 80 wt% fat and at
15 least 2.0 wt% of the ester mixture can be regarded as very
beneficial in that these not only have a very low fat
content, but also show a significant lowering of the blood
cholesterol working if applied similar to a common
butterlike spread on a regular, daily basis. This applies
20 even more for products with less than 60 wt% fat. In
another embodiment, the spread comprises at least 3 wt% of
the ester mixture. In a particular embodiment thereof, the
spread comprises at least 5 wt% of the ester mixture, the
ester mixture preferably being obtained by a process in
25 which the sterol ester (mixture) to be hydrolyzed comprises
oryzanol.

Another advantage of the present invention is that by
addition of the mixture obtained by esterification the
30 amount of hardstock required to make a spreadable product
out of above mentioned liquid oils can be reduced, thereby
optimizing the amount of PUFA rich glycerides in the
product.

35 The use of the ester mixture in cheese shows an additional
beneficial effect, which is that the cholesterol in cheese
is adsorbed by the body in a far lesser amount if the ester

mixture is present in the cheese than without. Hence, whereas cheese consumption usually adds cholesterol to the body, with the use of the ester mixture a strongly reduced absorption and a blood cholesterol lowering effect will be found. This effect can also be found for food products other than cheese comprising cholesterol or consumption of cholesterol containing food products in combination with the products as described in the invention. The amount of ester mixture to be added on the preparation of the cheese is preferably higher than the amount of cholesterol on a molar weight basis, so that the cheese product obtained still contains the ester mixture in a free and active form.

The fat that is applied in these fat based food products can be any fat, such as dairy fat and/or vegetable fat. However, if fat is present, for health reasons the use of one or more vegetable fat sources is preferred. In particular, the use of liquid fats is preferred. These can be hydrogenated, interesterified, and the like. The fat can be one single fat or a blend.

The use of fat compositions comprising a considerable amount of pufa rich triglycerides in addition to the use of the ester mixture is in particular considered highly beneficial. For example, oils of sunflower, safflower, rapeseed, linseed, linola and/or soybean can be used in a preferred embodiment.

If a fat blend is used, it is preferred that it comprises at least 30%, and more preferred at least 45% of polyunsaturated fatty acids, based on the total weight amount of the fat in the fat based food product. So, a strong effect on the cholesterol lowering effect is obtained if use is made of a mixture of the ester materials of the invention or these components in combination with the antioxidants like polyphenols and tocopherols, or extracts comprising all of these these components in a food product

in which a fat blend comprising at least 30 wt.% of pufa rich triglycerides is used.

The invention further envisages the use of an oil
5 concentrate comprising more than 4 wt% of the ester mixture for the preparation of a fat based food product as described above. In another preferred embodiment, the invention concerns the use of an oil concentrate comprising more than 4 wt% of the ester mixture for the preparation of
10 a yellow fat spread as described above.

Particular advantages are found when a fat based food product is prepared using 0.5-40 wt% of an ester mixture which is obtained by subjecting a mixture of
15 (a) 5-40 wt% campesterol
(b) 2-25 wt% β -sitosterol
(c) 20-60 wt% 24-methylene cyclo-artenol; and
(d) 10-50 wt% cyclo-artenol
to the process of the present invention, or, alternatively,
20 by using 0.5-40 wt% of an ester mixture which is obtained by subjecting a mixture of

- (a) 2-45 wt% alpha-amyrin
- (b) 0.2-25 wt% beta-amyrin
- (c) 0.2-35 wt% lupeol
- 25 (d) 2-45 wt% butyrospermol and
- (e) 0.1-15 wt% germanicol

to the process of the present invention.

In addition to the ester mixture as described above, food
30 products of the invention may comprise further ingredients which can have positive effects on the health. Examples of these are for example anti-oxidants e.g. polyphenols or tocopherols and tocotrienols.

35 The invention will now be further illustrated by means of the following examples:

Example IPreparation of the ester mixture

A crude soapstock is prepared by chemically refining rice
5 bran oil by addition of sodium lye (4M) followed by
isolating the aqueous bottom layer. The oil layer is washed
twice with water and the washing are combined with the
first washing.

10 The resulting crude soapstock contains per 500 ml:
12.5 g γ -oryzanol
5.5 g alkali salts of free fatty acids
32 g tri-acyl glycerols
surplus sodium lye
15 balance water

The γ -Oryzanol contained 4 esters of ferulic acid and the
following sterols:

24.6 wt% campesterol
20 15.5 wt% β -Sitosterol
31.2 wt% 24-methyl cycloartenol
28.7 wt% cycloartenol

The ester mixture can be obtained from the crude soapstock
25 as follows:

500 ml of crude soap stock can be concentrated to 100 ml by
evaporation, followed by the addition of 0.15 mol sodium
hydroxide dissolved in a small amount of water and 200 ml
30 ethanol. Upon boiling this mixture for about 2 hours, a
nearly complete saponification (over 90%) of the γ -Oryzanol
and the tri-acylglycerols will take place. After the
reaction is completed most of the ethanol can be removed by
evaporation.

35 The mixture is acidified by addition of 400 ml distilled
water containing approx. 0.3 mol hydrochloric acid. After

separation of the layers, the ferulic acid as well as the glycerol are collected in the aqueous bottom layer. The top layer containing the fatty acids and the sterols, can then be washed with water until neutral, The toplayer is then
5 dried by evaporating the water under vacuum (950C at 5 mm for about 1 h)

The resulting mixture of sterols and fatty acids can then be esterified by addition of methanol (4 times volume of
10 other methylsterol fraction) and 0.2 % of 96% sulfuric acid. The mixture is boiled for 2 hours, this results in the formation of methylesters of the fatty acids, while the sterols do not react. The lipid fraction of the mix is isolated and dried and contains the sterols and the
15 methylesters of fatty acids.

The esterification step of the sterols then takes place by adding 0.05 wt% of sodium methoxide and heating gradually to 1300C under vacuum (approx. 5 mm Hg) and constant
20 stirring, for about 2h, which results in interesterification of the methylesters with the sterols. The catalyst is destroyed by addition of some water. After phase separation the resulting esters of sterols and fatty acids can be isolated and subjected to molecular
25 distillation to remove the surplus of fatty acid methylesters.

The resulting ester mixture has the same ratio of components as indicated above.
30

Example II

Example I can be repeated whereby the hydrolisation of the crude soap stock takes place in an autoclave at 180 0C at 8
35 - 10 bar, and in the presence of a suitable catalyst.

Example III

The ester mixture obtained in example I can be incorporated in a 70% fat spread as follows:

44 parts refined sunflower oil (65% PUFA as linoleic acid)
5 was enriched with the ester mixture of example 1 and mixed with 20 parts of normal refined sunflower oil and 6 parts of a refined interesterified mixture of 50 parts fully hardened palm oil and 50 parts fully hardened palm kernel oil. To 70 parts of this fatblend, 0.1 part soybean
10 lecithin, 0.1 part monoglyceride and 0.1 part of β -carotene solution are added.

To 29 parts water, 0.3 part whey protein powder, a small amount of flavour, and citric acid to obtain a pH of 4.8
15 are added.

70 parts of the fat phase composition and 30 parts of the aqueous phase composition were mixed and kept at 60°C. The mixture was then passed through a Votator line with 2
20 scraped surface heat exchangers (A-units) and 1 stirred crystallizer (C-unit) in AAC-sequence operating at 800, 800 and 100 rpm respectively. The product leaving the C-unit had a temperature of 11°C. It was filled into tubs and stored at 5°C. A good and stable, high PUFA, high fat-
25 continuous spread enriched with 12% of the ester-mixture was obtained.

Example IV

30

Preparation of a spread 40%

35 parts refined sunflower oil (65% PUFA as linoleic acid) can be enriched with the ester mixture of example II and mixed with 5 parts of a refined interesterified mixture of
35 50 parts fully hardened palm oil and 50 parts fully hardened palm kernel oil. To 39 parts of this fatblend, 0.1 part soybean lecithin, 0.1 part monoglyceride and a small

amount of β -carotene solution are added.

To 57 parts water, 2 parts gelatine, 0.3 part whey protein powder, a small amount of flavour, preservative and citric acid to obtain a pH of 4.7 are added.

40 parts of the fat phase composition and 60 parts of the aqueous phase composition are mixed and kept at 60°C. The mixture can then be passed through a Votator line with 2 scraped surface heat exchangers (A-units) and 2 stirred crystallizers (C-unit), in ACAC-sequence operating at 500, 1000, 600 and 100 rpm respectively. The product leaving the last C-unit has a temperature of 10°C. It is filled into tubs and stored at 5°C. A good and stable, high PUFA, low fat-continuous spread enriched with 7% of the ester mixture is obtained.

Example V

20 Preparation of a dressing 33% fat

55 parts of water is mixed with 11 parts of various flavour components, preservatives, thickeners and emulsifiers. The mixture is thoroughly mixed in a stainless steel stirred vessel. To this aqueous mixture 33 parts of sunflower oil (65% PUFA as linoleic acid) enriched with the ester mixture as described in Example 1 and a 2% free phytosterol mixture is added, thoroughly mixed for an additional 15 min, to obtain a pre-emulsion. The pre-emulsion is brought into a colloid mill (Prestomill PM30) and processed at a split-size between level 15 and 20 and a throughput between level 4 and 6. A good and stable water continuous dressing enriched with 10% of the ester mixture and 2% free phytosterol mixture is obtained.

Claims

1. Process for the manufacture of a mixture of and ester
5 mixture comprising the steps of:
 - (a) hydrolysing a sterol ester or a mixture of sterol esters such that a mixture is obtained comprising phenolic and/or fatty acids, and free sterols; and
 - 10 (b) esterifying the so obtained free sterols with particular fatty acids.
2. Process according to claim 1, comprising the steps of
15 (a) hydrolysing a sterol ester or a mixture of sterol esters such that a mixture is obtained comprising phenolic acids and/or fatty acids, and free sterols; and
(b) separating the phenolic acids and/or fatty from the reaction mixture; and
20 (c) esterifying the so obtained free sterols with particular fatty acids.
3. Process according to any one of claims 1 or 2, wherein the sterol ester or mixture of sterol esters is a
25 mixture comprising phenolic acid sterol esters.
4. Process according to any one of claims 1-3, wherein the sterol ester or mixture of sterol esters consist of a phenolic acid sterol ester or a phenolic acid
30 sterol ester mixture.
5. Process according to any one of claims 1-4, wherein the sterol ester or sterol ester mixture is present in the reaction mixture at a concentration of 5 to 90 wt%
35
6. Process according to one or more of the preceding claims wherein the phenolic acid and or fatty acid

originating from the sterol ester starting material is separated from the reaction mixture prior to esterification.

- 5 7. Process according to one or more of the preceding claims wherein the sterols are esterified to C_{2-22} fatty acids sterol esters.
- 10 8. Process according to one or more of the preceding claims wherein the sterols are esterified to C_{8-22} fatty acids sterol esters.
- 15 9. Process according to one or more of the preceding claims wherein the sterols are esterified to fatty acid sterol esters of rice bran oil, sunflower, safflower, rapeseed, linseed, linola and/or soybean.
- 20 10. Process according to one or more of the preceding claims in which the starting material is obtained from rice bran.
- 25 11. Process according to any one one of claims 1-9 wherein the starting material is obtained from sheanut.
- 30 12. Mixture of fatty acid esters of sterols obtainable by any one of the processes of claims 1-11.
- 35 13. Mixture of fatty acid esters of sterols, characterized in that the mixture is obtained by subjecting a mixture of
 - (a) 5-40 wt% campesterol
 - (b) 2-25 wt% β -sitosterol
 - (c) 20-60 wt% 24-methylene cyclo-artenol; and
 - (d) 10-50 wt% cyclo-artenolto the process of any one of claims 1-11.

14. Mixture of fatty acid esters of sterols, characterized in that the mixture is obtained by subjecting a mixture of
- (a) 2-45 wt% alpha-amyirin
 - 5 (b) 0.2-25 wt% beta-amyirin
 - (c) 0.2-35 wt% lupeol
 - (d) 2-45 wt% butyrospermol and
 - (e) 0.1-15 wt% germanicol
- to the process of any one of claims 1-11.
- 10
15. Fat based food product comprising 0.5 to 40 wt% of an ester mixture obtainable by any one of the processes of claims 1-11.
- 15 16. Fat based food product according to claim 15 selected from the group of margarines, butter, spreads, mayonnaise, dressings, shortenings, cooking and frying oils.
- 20 17. Fat based food product according to claim 16, the product being selected from the group of margarines, butter and spreads.
- 25 18. Fat based food product according to any one of claims 15-17, characterized in that the product comprises 0.5-40 wt% of an ester mixture of any one of claims 13-14.

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